22.6.2015 Measurement study

MONDAY, 6/22

Pretreatment: Diluted plasmids for 10ul H20 and incubated in room temperature for 10 minutes. Liquid turned to red-colored. Mixed gently with pipette and transfered liquid into 0,5ml eppendorf (find names, companies and robustness). The markins of the DNA-tubes were AHD1, AHD2, AHD3 and AHD4.

Made SOC with the protocol = 49,5 ml SOB, 500ul $MgCl_2$ (1 M) and 1000ul Glucose (1 M). SOC to the autoclave.

Added 50 ul of ice cold compenent TOP10 cells into pre-chilled 2ml tube (find names, companies and robustness) and 2 ul of resuspended DNA to the same tube, and mixed with pipette gently. Liquid was transparent and there was some moisture on the tube walls. The cells were incubated on ice for 46 mins. The tubes were put in a water bath (42C) for 60s. After the heat shock the cells were on ice incubation for 5 minutes. Incubated the cells at 37C for 1 hr with shaking (230 RPM). At this point there seemed to be growth in tubes, because the liquid turned to be bleary.

Two petri disher were labeled for each transformation, 2 of them with AMP (AHD4) and 6 of them with chloramphenicol (AHD1, AHD2 and AHD3). The incubation of plates (37C, 230 RPM) started at 17:30 and ended 9:00 for 15 hr 30 min period with .

24.6 Measurement study

WEDNESDAY, 6/24

Petra

Did o/n cultures of AHD1, AHD2, AHD3 and AHD4

- 1 colony from each plate (there were 2 plates for each transformation)
- colonies used are marked to the plates
- 2 ml cultures, LB media
- Culture tube details can be seen from the box stored in the storage room
- Antibiotic concentrations in cultures
    - Chloramphenicol: 25 ug/ml: 1,5 ul CHL to AHD1, AD2 and AHD3
    - Ampicillin: 50 ug/ml: 0,8 ul AMP to AHD4
- -> 8 o/n tubes in total

Cultures grown at 37 C with shaking (230 rpm)

Incubation started at 13.50 and ended 10.30

25.6.2015 Measurement study

THURSDAY, 6/25

Started making purifications for the plasmids from the previous day overnight cultures (2ml with antibiotics) (AHD1 A and B; AHD2 A and B; AHD3 A and B; AHD4 A and B).
First prepared NucleoSpin Plasmid EasyPure buffers by adding the content of RNase-liquid into Resuspension Buffer A1 and 100 ml of ethanol (96 v-%) for Wash Buffer AQ.

The overnight cultures were transfered into 2 ml eppendorf-tubes and the cells were pelleted with the eppendorf-centrifuge (Eppendorf AG Inc. Hamburg, Centrifuge 5418, 12000 rpm, 2 mins). Added 250 ul Resuspension buffer A1 and vortexed the mixture until the cells resuspended. Added 250 ul Lysis buffer A2 (Sodium hydroxide solution 0,5-2,0 %) and inverted the tubes 5 times, which transferred liquid to blue-colored. Incubated 2 mins at room temperature for cell lysis, and added 350 ul Neutralization Buffer A3 (Guanidine hydrochloride 36-50 %) and inverted the tubes until the liquid was colorless. Buffer produced mucous sediment in the liquid, which seemed to be dead cell's remains. Centrifuged for 3 mins at 12000 RPM to pellet precipitate. Loaded clear supernatant onto the NucleioSpin Plasmid EasyPure Columns which were put into collection tubes (2 mL, Machenery-Nagel Inc., plastic information not available). Centrifuged for 30 s at the speed of 8000 rpm and discarded the flow-through. Added 450 ul Wash Buffer AQ to the spin column, and centrifuged for 1 min at the speed of 12000 rpm until the spin column was dry. Added 50 ul Elution Buffer AE (5 mM Tris/HVl, pH 8,5) onto the middle of the column and incubated for 1 min. Centrifuged the mixture for 1 min at the speed of 12000 rpm, and the flowthrough was transparent. The purified liquid was measurede by NanoDrop.

The following results were obtained from NanoDrop Lite (Thermo Scientific Inc.), shown in table 1.

Table1

Sample	DNA (ng/µl)	Absorbance (A260/A280)
AHD1 (J23101) 1#	93,5	1,83
AHD1 (J23101) 2#	97,3	1,87
AHD2 (J23106) 1#	46,6	1,90
AHD2 (J23106) 2#	51,1	1,93
AHD3 (J23117) 1#	48,8	1,91
AHD3 (J23117) 2#	53,0	1,92
AHD4 (I13504) 1#	124,6	1,85
AHD4 (I13504) 2#	167,0	1,85

26.6.2015 Measurement study

FRIDAY, 6/26

Started restriction:

Markings in tubes: AHD1 R, AHD2 R, AHD3 R, AHD4 R and AHD5 R.
Kept all enzymes and buffers on ice if the protocol didn't expect anything else. Added 2,5ul 10x NEB CutSmart Buffer into the tube (material, dimensions, manufacturer. In order to get 250ng DNA for mixtures, the following volumes of purified plasmids were added to tube shown in Table 1 and the total liquid volume of the tube was balanced to be 25 ul with sterile water, after the enzymes were pipetted. Added 0,5ul of each restriction enzyme following Table 1. Mixed by pipetting and spinned the samples to get reagents in the bottom of tubes.

Incubated samples at 37C for 30 min and inactivated the restriction enzymes at 80C for 20 mins.

Table1

Sample	DNA (ng/µl)	DNA volume added (ul)	Ion-free water added (ul)	Restriction enzyme used
AHD1 (J23101)	93,5	2,67	18,83	EcoRI & SpeI
AHD2 (J23106)	46,6	5,36	16,14	EcoRI & SpeI
AHD3 (J23117)	48,8	5,12	16,38	EcoRI & SpeI
AHD4 (I13504)	124,6	2,01	19,49	XbaI & PstI
AHD5 (pSB1C3)	25,0	10,0	11,5	EcoRI & PstI

Ligation:

Markins in tubes: 145 LM, 245 LM and 345 LM,

where the first 3 numbers indicate what samples were ligated and "LM" means "Ligated, Measurement"

Kept all enzymes and buffer.Added 3x2ul of restricted DNA, 2ul of 10x T4 DNA ligase buffer and 0,2 ul T4 DNA ligase to each tube and balanced total volume to be 20 ul with nuclease-free water. There seemed to be some white sediment in restriction sample AHD1, tried to avoid that when pipetting the restricted DNA-liquid. After the addition of ligase, there was 20 min pause when the tubes were in ice before starting incubating 30 mins at 22C. The ligase was inactivated at 65C for 10 mins and put on ice.

Stored the ligations in -20°C.